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B vitamins relieve neuropathic pain behaviors induced by infraorbital nerve constriction in rats

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ABSTRACT

Aims: There is mounting evidence that use of B vitamins can help control neuropathic pain. This study investigated if treatment with B1, B6 and B12 vitamins, alone or in combination with carbamazepine, can ameliorate distinct nociceptive behaviors in a model of trigeminal neuropathic pain.
Main methods: Male Wistar rats were submitted to infraorbital nerve constriction or sham surgery and received a 5-day treatment with one of the B vitamins, a single carbamazepine injection or the association of both treatments and were tested for facial thermal and mechanical hyperalgesia at different time intervals.
Key findings: Repeated treatment with B1 (thiamine), B6 (pyridoxine) and B12 (cyanocobalamin) vitamins (at 180, 180 and 18 mg/kg/day, respectively, for 5 days) prevented the development of heat hyperalgesia after infraorbital nerve injury, but only B12 and B6 treatments attenuated cold and mechanical hyperalgesia, respectively. A single injection of carbamazepine (30 mg/kg) significantly reduced thermal, but not mechanical, hyperalgesia after nerve injury. Combinations of lower doses of each B vitamin (B1 and B6 at 18 mg/kg/day and B12 at 1.8 mg/kg/day for 5 days) with carbamazepine (10 mg/kg) markedly reduced heat hyperalgesia after infraorbital nerve injury. Treatment with B12 (1.8 mg/kg/day) combined with carbamazepine (10 mg/kg) also synergized to attenuate cold hyperalgesia at some time points, but combination of B6 (18 mg/kg/day) with carbamazepine (30 mg/kg) failed to modify mechanical hyperalgesia.
Significance: We suggest that B vitamins might constitute a relevant adjuvant to control some aspects of the pain afflicting patients suffering from trigeminal neuropathic pain.

Introduction

Chronic neuropathic pain caused by lesions in the peripheral or central nervous system comes in various forms which are still difficult to control and treat. Trigeminal neuralgia, continues to represent a therapeutic challenge as its pathophysiology is not yet fully understood (Adams, 1989; Alves et al., 2004; Chichorro et al., 2006a, 2006b; Cuellar et al., 2010; Eide and Rabben, 1998; Fisher et al., 2003; Kleef et al., 2009; Sindrup and Jensen, 2002; Zakrzewska, 2004). Currently, anticonvulsant drugs are commonly used to control symptoms in neuropathic pain, especially for their ability to reduce neuronal excitability by acting through different mechanisms (Attal et al., 2010; Delzell and Grelle, 1999; Finnerup et al., 2010a, 2010b; Gill et al., 2011; Martin and Forouzanfar, 2011; Willimann, 2011; Zakrzewska and McMillan, 2011). The most studied agents are carbamazepine, gabapentin and pregabalin, but lamotrigine, topiramate and oxcarbazepine have also shown analgesic potential in different pain models and in clinical studies (Ambrósio et al., 2002; Cheng and Chiou, 2006; Cheshire, 2002; Czapinski et al., 2005; Lynch and Watson, 2006; Siniscalchi et al., 2011; Wilhelmus and Forouzanfar, 2011). Indeed, carbamazepine is currently the first choice for treatment of trigeminal neuralgia, as it has been shown to reduce pain symptoms in about 70% of the cases, and it is also used to diagnose the condition (Ambrósio et al., 2002; Kleef et al., 2009). However, continuous treatment with carbamazepine or other anticonvulsant drugs results in numerous adverse effects such as drowsiness, dizziness, ataxia, diplopia, blurred vision, nausea, dry mouth, constipation and diarrhea (Alves et al., 2004; Attal et al., 2010; Zakrzewska and Patsalos, 1992). In addition, many patients submitted to repeated carbamazepine treatment become refractory to the medication (Attal et al., 2010; Boto, 2010; Bergouignan, 1970; Zakrzewska and Patsalos, 1992). Thus, additional therapeutic options are in high demand (Boto, 2010; Zakrzewska et al., 2005a, 2005b).

Thiamine, pyridoxine and cyanocobalamin (i.e. B1, B6 and B12 vitamins) are the major vitamins of B complex. They act mainly as coenzymes of different reactions, participating importantly in the metabolism of carbohydrates, proteins and lipids. They also play an important role in the formation of new cells, DNA, RNA and myelin (Dakshinamurti et al., 2003; Depeint et al., 2006a, 2006b; Healton et al., 1991; McCombe and McLeod, 1984; Selhub et al., 2010). According to some studies, B vitamins present neuroprotective effects, which

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are suggested to be related to their analgesic action in various models of neuropathic pain (Jolivalt et al., 2009; Mixcoatl-Zecuatl et al., 2008; Reyes-Garcia et al., 2004; Wang et al., 2005; Zimmerman et al., 1990). However, to the best of our knowledge, the influence of the vitamins of B complex on orofacial pain has never been investigated. Thus, the present study aimed to evaluate the influence of the administration of B1, B6 and B12 vitamins of the B complex, either delivered alone or combined with carbamazepine treatment, in thermal and mechanical hyperalgesia in a model of trigeminal neuropathic pain induced in rats by constriction of the infraorbital nerve.

Materials and methods

Animals

Experiments were conducted on male Wistar rats weighing 180–220 g, maintained five to a cage at controlled temperature (22 ± 1 °C) under a 12/12 h light/dark cycle (lights on at 08:00 h) with free access to chow and tap water. They were acclimatized to the laboratory for at least 48 h before use. The experimental procedures were previously approved by UFPR's institutional Committee on the Ethical Use of Animals (authorization # 471), where the study was carried out, and conducted in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and Brazilian regulations on animal welfare. All efforts were made to minimize the number of animals used and their suffering.

Constriction of the infraorbital nerve

The constriction of the infraorbital nerve (CION) was performed according to the slight modification of the procedure originally proposed by Vos et al. (1994) described by Chichorro et al. (2006a). Briefly, rats were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine and xylazine (50 and 10 mg/kg, respectively) and an incision was made in the skin of the snout, under the right eye, about 3 mm caudal to the mystacial pads. The superior lip elevator and anterior superficial masseter muscles were bluntly dissected to expose the rostral end of the infraorbital nerve, as it emerged from the infraorbital fissure. Special care was taken to not damage the facial nerve. Two silk 4-0 ligatures were then tied loosely and 2 mm apart around the infraorbital nerve and the wound was closed with additional silk sutures (4-0). Sham-operated rats were treated identically, but no ligatures were applied to the infraorbital nerve. After surgery, all rats were maintained in a warm room until they recovered from anesthesia.

Cold stimulation

Before each testing session, animals were placed in individual plastic observation cages and left to adapt to the environment for at least 30 min. After this period, they usually displayed considerable sniffing, but very little locomotor activity. Cold stimulation was applied by the experimenter in the form of a brief 1-s spray of tetrafluoroethane to the center of the right vibrissal pads, while gently restraining the animal in its cage by placing one hand around its trunk. The total duration of bilateral facial grooming behavior with both forepaws directed to the snout was recorded, using a stopwatch, over the first 2 min following application of the cold stimulus, as an index of the intensity of nocifensive responsiveness. In rats subjected to CION, cold responsiveness was evaluated before and on days 2, 4, 6, 9 and 12 after surgery. It is important to point out that cold hyperalgesia peaks on days 4 to 6 after CION surgery, as previously reported by our group (Chichorro et al., 2006a).

Heat stimulation

Thermal hyperalgesia on the orofacial area was measured as previously described (Chichorro et al., 2009). On each occasion, the animal was temporarily removed from its home cage and gently held by the experimenter and a radiant heat source was positioned 1 cm from the surface of the right vibrissal pad. The latency to display either head withdrawal or vigorous flicking of the snout was recorded (in s) using a stopwatch, and a 20 s cut-off time was used to prevent tissue damage. Reaction to heat stimulation was evaluated before (basal responsiveness) and on days 2, 4, 6, 9 and 12 after CION surgery. It is important to point out that heat hyperalgesia peaks on days 4 to 6 after CION surgery, as previously reported by our group (Chichorro et al., 2009).

Mechanical stimulation

To assess orofacial mechanical hyperalgesia, before each testing session the animals were placed in individual plastic cages and left to adapt to the environment for at least 2 h. The mechanical threshold was measured using a graded series of 8 von Frey monofilaments ranging from 0.04 to 8 g (Semmes–Weinstein monofilaments, Stoelting, Wood Dale, IL) as described previously (Christensen et al., 1999; Chichorro et al., 2006b). Each monofilament was applied near the center of the right vibrissal pad, to the point of bending, three times on the nerve-injured side. Each stimulation series began with the filament producing the lowest force, and proceeded up to the filament that evoked one of the following nocifensive behaviors twice: brisk head withdrawal, escape or attack reactions, or short-lasting facial grooming. Only rats that did not react to application of the 8-g filament in the preoperative tests were included in this study, to avoid unspecific responses. It is important to point out that mechanical hyperalgesia starts to develop around 10 days after CION surgery, as previously reported by our group (Chichorro et al., 2006b).

Drugs

B1, B6 and B12 vitamins (thiamine, pyridoxine and cyanocobalamin, respectively) were obtained from Galena Química e Farmacêutica (Campinas, SP, Brazil) and were all dissolved in sterile saline solution. Ketamine was obtained from Rhobifarma Ind. Farmacêutica (Hortolândia, SP, Brazil) and xylazine from Laboratórios König S.A. (Avellaneda, Argentina). Carbamazepine was purchased from Sigma (St. Louis, MO, USA) and was dissolved in saline solution containing 10% DMSO, 1% ethanol and 1% Tween 80.

Experimental protocols

To evaluate the influence of B vitamins on the thermal (heat and cold) hyperalgesia induced by infraorbital nerve constriction, thiamine, pyridoxine and cyanocobalamin were administrated individually at 180, 180 and 18 mg/kg (s.c., 1 mL/kg), respectively, soon after CION surgery (day 0) and then again daily throughout the next 4 consecutive days (day 1 through day 4 post-surgery). Control animals were treated identically with saline (1 mL/kg, s.c.). Responsiveness to cold and heat stimulation was assessed before and on days 2, 4, 6, 9 and 12 after CION surgery. When behavioral testing coincided with days of injection (i.e. days 2 and 4 after CION surgery) responsiveness was assessed both prior to and then again at 1 h after the injection of the day, but on the other days responsiveness was assessed only once. To evaluate the influence of B vitamins on the mechanical hyperalgesia, rats received thiamine, pyridoxine and cyanocobalamin individually, at the same doses selected for the thermal hyperalgesia experiments, but the treatments started on day 8 after CION surgery and were repeated daily until day 12. The mechanical threshold of rats was assessed before the surgery and on days 8, 10,

12, 16 and 20 after the surgery, but when test sessions were conducted during the treatment period (i.e. days 8, 10 and 12) responsiveness was first assessed prior to injection and then again at 1 h after treatment of the day. The doses of B1 and B6 vitamins were chosen based on the study of Jolivalt et al. (2009). The dose of B12 employed was 10 times lower than that of the other vitamins, in agreement with experimental and clinical studies, which used B12 at different, but always lower dose ratios compared to B1 and B6 (Allen, 2010; Caram-Salas, et al., 2004; Depeint et al., 2006a, 2006b; Jolivalt et al., 2009; Mauro et al., 2000; Mibielli et al., 2009). Additional groups of rats were treated with single injections of either B1, B6 or B12 (180, 180 and 18 mg/kg, respectively, s.c.), or vehicle (1 mL/kg, s.c.), carbamazepine (10 or 30 mg/kg, i.p.) or the corresponding vehicle (1 mL/kg, i.p.) only on day 4 after CION surgery and thermal hyperalgesia (to heat and/or cold stimuli) was assessed prior to injection and also at 1-h intervals up to the fourth hour thereafter. To evaluate effects of carbamazepine on mechanical hyperalgesia, on day 20 after CION surgery the rats received carbamazepine (30 mg/kg, i.p.) or vehicle (1 mL/kg, i.p.) and responsiveness to mechanical stimulation of the face was assessed prior to treatment and then again at 1-h intervals up to 4 h later. To investigate the effects of association of different B vitamins with carbamazepine on thermal (heat and/or cold) hyperalgesia, B1, B6 and B12 vitamins were administered individually at lower doses (18, 18 and 1.8 mg/kg/day, respectively, s.c.), starting soon after CION surgery (day 0) and repeated on the following 4 days (days 1 through 4). On day 4, immediately after the last vitamin administration, the rats also received a single i.p. administration of carbamazepine (10 mg/kg) and heat and/or cold hyperalgesia was assessed at 1 h-intervals up to the sixth hour. In addition, to investigate the effect of association of B6 with carbamazepine on mechanical hyperalgesia, the vitamin was administered individually at 18 mg/kg/day, starting on day 8 after CION surgery and repeated on the following 4 days (days 8 through 12). On day 12, immediately after the last vitamin administration, the rats also received a single i.p. administration of carbamazepine (30 mg/kg) and mechanical hyperalgesia was assessed at 1-h intervals up to the sixth hour. It is noteworthy that different groups of rats were tested for cold, heat or mechanical hyperalgesia experiments.

Statistical analysis and presentation of data

All data are presented as mean ± S.E.M. (standard error mean) and in instances where error bars of certain values do not appear in the figures, it is because they were smaller than the symbol depicting the corresponding mean value. Two-way repeated measures ANOVA was used to analyze the data from both thermal and mechanical stimulation, with drug treatment as the independent factor and the different evaluation time points of nociceptive behavior as the repeated measure. In case of significant differences with the independent factor or with the interaction between the independent and repeated factors, one-way ANOVA followed by the Duncan's post-hoc test, was performed. In all statistical analysis, P values less than 0.05 were considered significant.

Results

As previously reported by our group, CION surgery leads to the development of orofacial sensory alterations in rats which include hyperalgesia to cold and heat stimuli, with similar time courses (Chichorro et al., 2006a, 2009), and a more delayed facial mechanical hyperalgesia that only starts 10 days after the surgery (Chichorro et al., 2006b). The present study assessed the effects of repeated treatment with B1, B6 or B12 vitamins, alone or combined with a single injection of carbamazepine, on changes in behavioral responses to thermal and mechanical stimulation caused by CION injury. As illustrated on Fig. 1, daily treatment with B1 or B6 vitamins (both at 180 mg/kg/day, s.c.) from day 0 to day 4 abolished the development

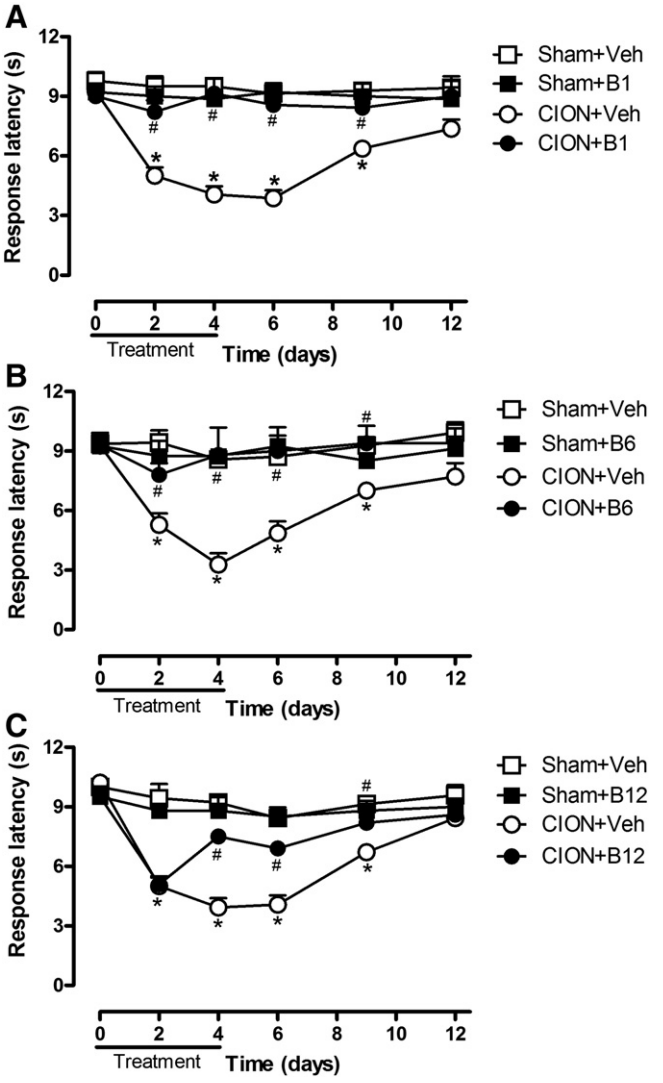

Fig. 1. Influence of repeated treatment with B vitamins on heat hyperalgesia induced by CION surgery. Rats were treated daily with vitamin B1 (A), B6 (B) or B12 (C), at 180, 180 or 18 mg/kg/day, respectively, for 5 days, starting on the day of CION surgery. Heat hyperalgesia was assessed before (day 0) and on days 2 and 4, one hour after the treatments, and on days 6, 9 and 12 after surgery. Values represent means ± SE mean of 5–8 rats. * and # indicate $P<0.05$ when compared to corresponding value of sham- and CION-operated rats treated with vehicle, respectively (Two way ANOVA followed by Duncan test).

of heat hyperalgesia in CION-injured rats compared with the control vehicle-treated CION group (Fig. 1A and B). Moreover, vitamin B12 (at 18 mg/kg/day, s.c.) treatment also reduced orofacial heat hyperalgesia, having displayed marked antihyperalgesic effects on days 4 and 6 (but not day 2) after surgery, and a full reversal of the hyperalgesia on day 9 (Fig. 1C). On the other hand, when single injections of these vitamins were given on day 4 after CION surgery, those same doses of B1, B6 and B12 each failed to influence ongoing heat hyperalgesia for up to 4 h after administration (Fig. 2). Although rats submitted to CION surgery also manifested orofacial cold hyperalgesia with a time course similar to than seen for heat hyperalgesia, daily treatment with B1 or B6 vitamins, at the same doses and over the same period mentioned above, failed to modify the development or to attenuate orofacial cold hyperalgesia (Fig. 3A and B). However, treatment with B12 (18 mg/kg/day, for 5 days) reduced cold hyperalgesia on days 6 and 9 compared to vehicle-treated CION rats (Fig. 3C). Interestingly, orofacial mechanical hyperalgesia after CION was significantly reduced throughout days 10 to 20 by treatment with vitamin B6 (Fig. 4B), whereas B1 and B12 were ineffective in altering

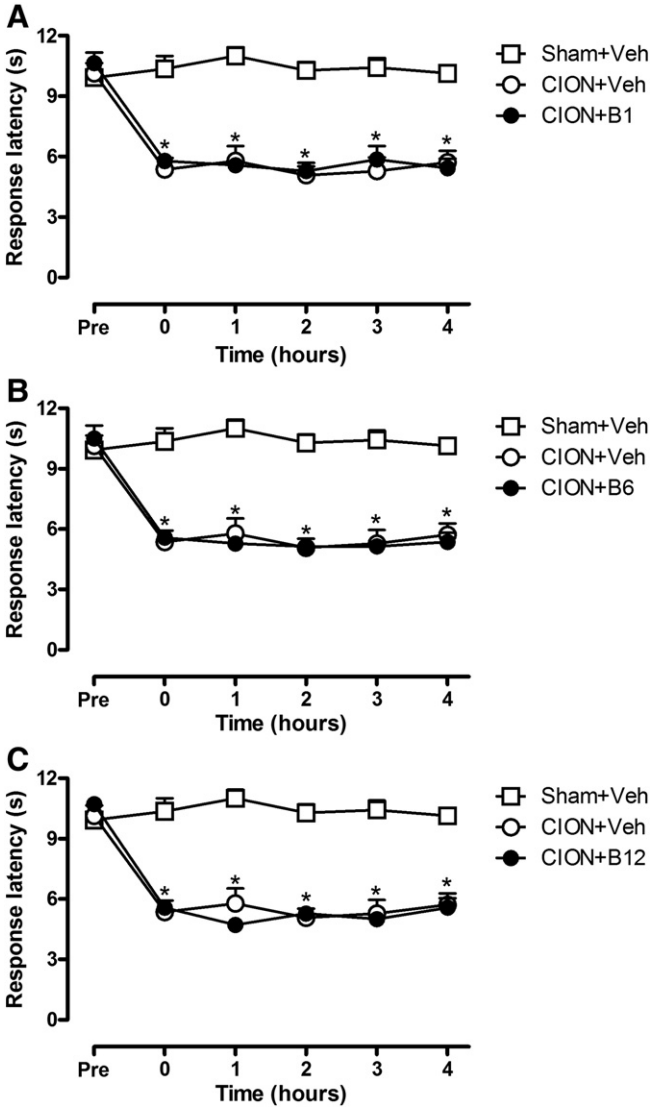

Fig. 2. Influence of a single injection of vitamin B1, B6 or B12 on established CION-induced heat hyperalgesia. Basal heat responsiveness was assessed before CION surgery (Pre) and then again on day 4 after surgery, first prior to (0 h) and then at each hour after s.c. injection of vitamin B1 (A), B6 (B) or B12 (C), at 180, 180 or 18 mg/kg, respectively. Values represent means ± SE mean of 7 rats. * indicate $P<0.05$ when compared to corresponding value of sham-operated rats treated with vehicle (Two way ANOVA followed by Duncan test).

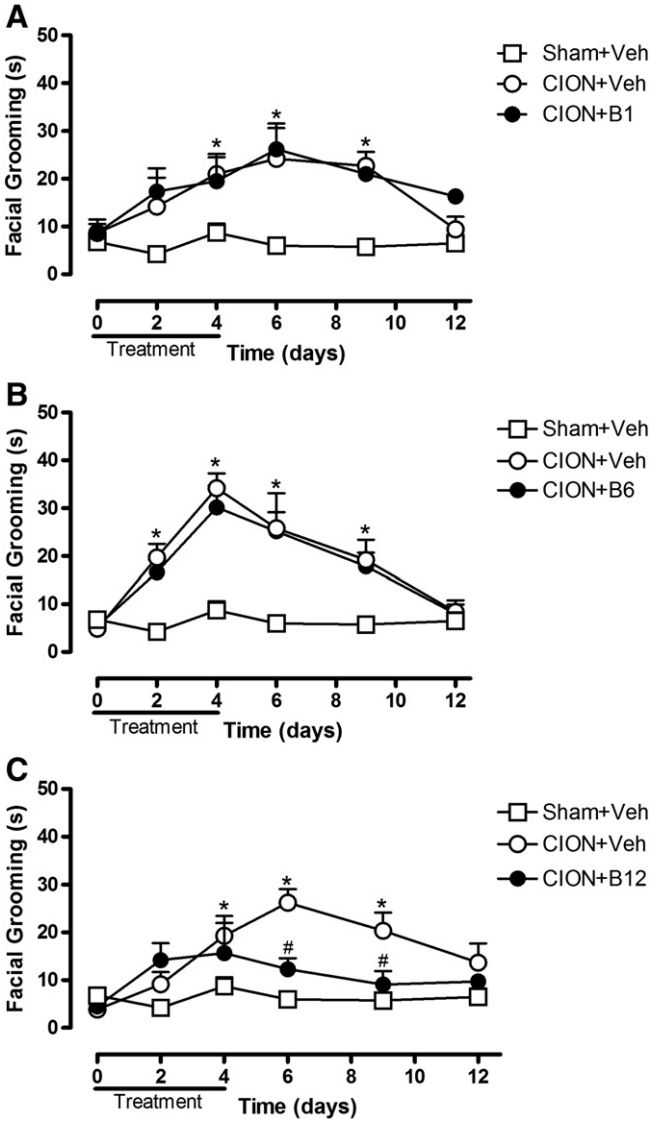

Fig. 3. Influence of repeated treatment with B vitamins on cold hyperalgesia induced by CION surgery. Rats were treated daily with vitamin B1 (A), B6 (B) or B12 (C), at 180, 180 or 18 mg/kg/day, respectively, for 5 days, starting on the day of CION surgery. Heat hyperalgesia was assessed before (day 0) and on days 2 and 4, one hour after the treatments, and on days 6, 9 and 12 after surgery. Values represent means ± SE mean of 5–7 rats. * and # indicate $P<0.05$ when compared to corresponding value of sham- and CION-operated rats treated with vehicle, respectively (Two way ANOVA followed by Duncan test).

mechanical sensory threshold at all time points evaluated (Fig. 4A and C).

Considering that carbamazepine is the first choice treatment for trigeminal neuralgia, we also investigated its effects on sensory alterations induced by CION. Single carbamazepine (30 mg/kg, i.p.) injection on day 4 after surgery reduced heat hyperalgesia significantly from 2 to 4 h after administration, but at 10 mg/kg the anticonvulsant was ineffective (Fig. 5A). Considering that CION-induced heat hyperalgesia was amenable to the inhibition by each of the B vitamins tested and to carbamazepine, we next assessed the influence of combinations of lower doses of B1 (18 mg/kg/day), B6 (18 mg/kg/day) or B12 (1.8 mg/kg/day) for 5 days, together with a low dose of carbamazepine (10 mg/kg) on day 4 after CION, in order to detect a possible synergistic effect. Repeated daily treatment with vitamin B1 or B6 alone resulted in discrete reductions of heat hyperalgesia on day 4 which lasted up to 2 h (Fig. 5B and C), but heat hyperalgesia at that time point was not influenced by a similar treatment with B12 (Fig. 5D). In sharp contrast, when treatments with B1, B6 or B12 were combined on day 4 after CION with a dose of carbamazepine that was ineffective per se, each of the combinations promoted a more robust and persistent reduction (up to 5 h) in heat hyperalgesia compared with each drug treatment alone, reflecting a synergistic effect (Fig. 5B,C and D).

Cold hyperalgesia on day 4 after CION injury was unchanged by a single injection of carbamazepine at 10 mg/kg, but a pronounced antihyperalgesic effect was detected at the first and second hours following its administration at 30 mg/kg (Fig. 6A). Repeated 5-day treatment with the lower dose of vitamin B12 (1.8 mg/kg) attenuated the cold hyperalgesia at 1 and 2 h after the last injection, whereas the combination of vitamin B12 treatment with an injection of carbamazepine (10 mg/kg) on day 4 after CION abolished cold hyperalgesia at 1 h and also promoted significant reductions at 2 and 4 h (Fig. 6B). At variance with the results obtained in relation to thermal hyperalgesia, mechanical hyperalgesia on day 20 after CION was fully resistant to alleviation either by a single injection of carbamazepine (30 mg/kg; Fig. 7A), or by administration of the anticonvulsant (also at 30 mg/kg) in combination with repeated

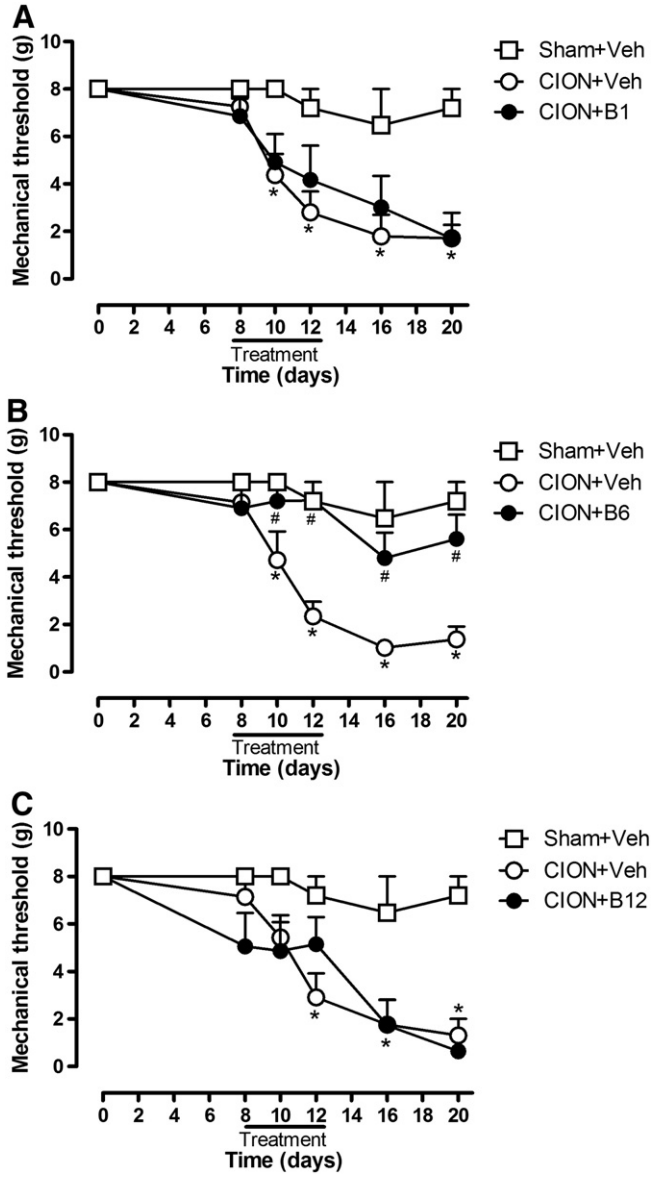

Fig. 4. Influence of repeated treatment with B vitamins on mechanical hyperalgesia induced by CION surgery. Rats were treated daily with vitamin B1 (A), B6 (B) or B12 (C), at 180, 180 or 18 mg/kg/day, respectively, for 5 days, starting on day 8 after CION surgery. Mechanical hyperalgesia was assessed before (day 0) and on days 8, 10, 12, 16 and 20 after CION surgery. Values represent means ± SE mean of 5–8 rats. * and # indicate $P<0.05$ when compared to corresponding value of sham- and CION-operated rats treated with vehicle, respectively (Two way ANOVA followed by Duncan test).

5-day treatment with the lower dose of vitamin B6 (18 mg/kg/day), on day 12 after surgery (Fig. 7B).

Discussion

Vitamins of the B complex have shown efficacy in controlling inflammatory or neuropathic pain in various animal models (Bartoszyk and Wild, 1989; Caram-Salas et al., 2006; França et al., 2001; Jolivalt et al., 2009; Mixcoatl-Zecuatl et al., 2008) and in patients (Abbas and Swai, 1997; Medina-Santillan et al., 2004; Simeonov et al., 1997). The current study shows, for the first time, that repeated systemic treatment for 5 days with vitamins B1, B6 or B12 can alleviate some of the nociceptive changes inflicted by CION surgery, an animal model of trigeminal neuropathic pain. Whereas all three vitamins were effective in reducing CION-induced heat hyperalgesia, cold and mechanical hyperalgesia were amenable to suppression only by vitamins B12 and B6, respectively. In addition, both types of thermal hyperalgesia were also susceptible to inhibition by a single injection of carbamazepine, but mechanical hyperalgesia was not. Importantly, at doses insufficient to cause effects on its own, carbamazepine synergized with low doses of each of the 3 vitamins to reduce heat hyperalgesia, and with vitamin B12 to limit cold hyperalgesia, but no synergism towards suppression of mechanical hyperalgesia was detected when combined with vitamin B6.

Previous studies on the effects of B vitamins on neuropathic pain employed different experimental models and evaluated changes in responses evoked by nociceptive stimuli of distinct sensory modalities. To facilitate comparisons between previously reported findings in other models of neuropathy and those we obtained in the model of CION-induced neuropathic pain, the results concerning the effects of vitamin B treatment on heat hyperalgesia will be discussed before those related to cold hyperalgesia and mechanical hyperalgesia.

Hind paw heat hyperalgesia induced by spinal ganglia compression or by loose ligation of the sciatic nerve in rats is reduced by single i.p. injection of vitamins B1, B6 and B12, alone or in combination (Song et al., 2009; Wang et al., 2005). The antihyperalgesic effect of the combination was significantly greater than that seen for individual vitamins, and repeated treatment with the combination (Wang et al., 2005) or B1 alone (Song et al., 2009) promoted long-term suppression of heat hyperalgesia. In the current study, repeated administration of each of the three vitamins was found to alleviate orofacial heat hyperalgesia promoted by CION surgery. Vitamins B1 and B6 each provided full suppression at the highest doses tested and partial reduction at 10-fold lower doses, whereas vitamin B12 only caused partial reduction at the highest dose tested. It remains to be tested if combined treatment with all 3 vitamins would result in better control of heat hyperalgesia in this model of trigeminal pain. However, as single injections of vitamins B1, B6 and B12 each failed to acutely modify heat hyperalgesia, relief of hyperalgesia appears to require their repeated administration.

Effects of B vitamin treatment on cold hyperalgesia associated with models of neuropathic pain had not yet been examined. The current study shows that repeated injections of vitamin B12 were effective in reducing the orofacial cold hyperalgesia induced by CION surgery. However, neither vitamin B1 nor B6 treatments modified the intensity of behavioral responses to application of cold stimuli to the snout.

Mechanical hyperalgesia of the hind paw induced by spinal ganglia compression or loose sciatic nerve ligation in rats were reported to be resistant to acute administration of vitamins B1, B6 and B12, at doses which suppressed heat hyperalgesia (Wang et al., 2005). Conversely, in rats submitted to L5/L6 spinal nerve ligation, mechanical hyperalgesia has been shown to be alleviated by acute administration of vitamin B12 in dose-dependent fashion (Granados-Soto et al., 2004), or by single or combined treatment with vitamins B1, B6 and B12 (Caram-Salas et al., 2006; Reyes-Garcia et al., 2003, 2004). In this same model, oral administration of the vitamin B1 derivative benfotiamine or vitamin B12 each reduced mechanical hyperalgesia (Mixcoatl-Zecuatl et al., 2008). Streptozotocin-induced diabetic rats already presenting established hind paw mechanical hyperalgesia displayed dose-related reductions in this response following combined treatment with vitamins B1, B6 and B12 at 3 different dosage levels (Jolivalt et al., 2009). The present study revealed that orofacial mechanical hyperalgesia induced by CION was reduced by repeated injections of vitamin B6 alone for 5 days, but vitamins B1 or B12 were ineffective in this regard, even though the B1 vitamin dose was identical to the higher dose tested by Jolivalt et al. (2009).

The differences in vitamin B dosage and treatment regimens (acute/repeated, alone/combined) used in the above mentioned studies render it difficult to draw firm conclusions as to their antihyperalgesic activity, especially as they were obtained in various models of neuropathy. Overall, the limited evidence available regarding inhibition of

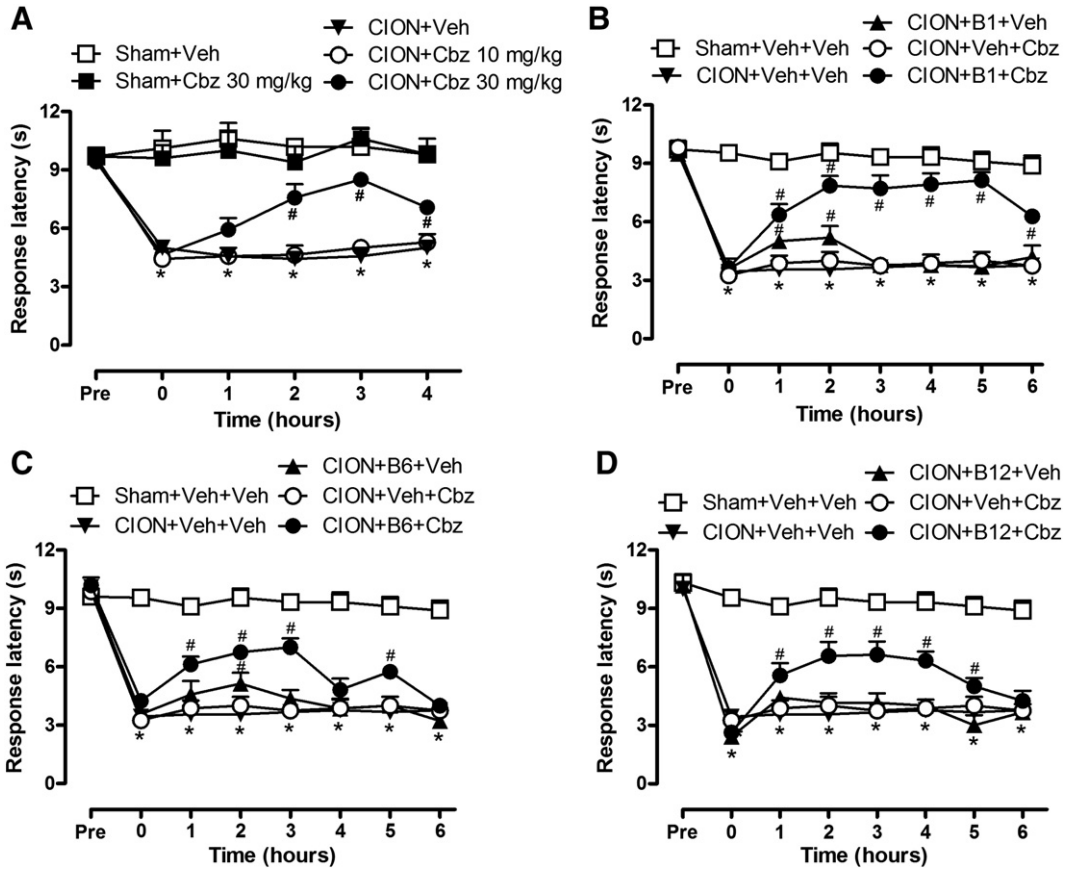

Fig. 5. Effect of carbamazepine alone or in combination with B vitamins on heat hyperalgesia induced by CION surgery. Basal heat responsiveness was assessed before CION surgery (Pre) and then again on day 4 after surgery, first prior to (0 h) and then at each hour after the injections of that day (see below). On day 4 after CION, rats received a single injection of carbamazepine (10 and 30 mg/kg, i.p.) or vehicle (1 mL/kg, i.p.; panel A). In the subsequent panels, rats were treated daily with vehicle (saline, 1 mL/kg, s.c.) or vitamin B1 (B), B6 (C) or B12 (D), at 18, 18 or 1.8 mg/kg/day, respectively, for 5 days, starting on the day of CION surgery and on day 4 the last injection was given simultaneously with injection of either vehicle (1 mL/kg, i.p.) or carbamazepine (10 mg/kg, i.p.). Values represent means ± SE mean of 5–9 rats. * and # indicate $P<0.05$ when compared to corresponding value of sham- and CION-operated rats treated with vehicle, respectively (Two way ANOVA followed by Duncan test).

hind paw or orofacial heat hyperalgesia seems to be consistent in demonstrating beneficial effects of all three B vitamins. However, there seem to be appreciable differences in the susceptibility of the mechanical hyperalgesia associated to different models to suppression by vitamins B1, B6 and/or B12. On the other hand, considering that these vitamins differentially affect hyperalgesia to heat, cold and mechanical stimuli in the CION-induced model, the mechanisms through which they suppress neuropathy-induced nociceptive sensory changes appear to be diverse.

Carbamazepine, the primary drug of choice for treatment of trigeminal neuralgia, reduced established CION-induced thermal hyperalgesia at 30 mg/kg, but mechanical hyperalgesia was unaffected. On the other hand, mechanical hyperalgesia induced by partial sciatic nerve ligation in rats or mice is reduced by this dose of the drug (Walker et al., 2003), while 100 mg/kg suppressed that caused by spinal nerve ligation in rats (Mixcoatl-Zecuatl et al., 2008), but this higher dose was found to promote sedation and ataxia in our rats (unpublished observations). Importantly, marked synergism towards inhibition of heat hyperalgesia was observed on day 4 after CION surgery when only 10 mg/kg of carbamazepine was combined with 10-fold lower doses of vitamins B1, B6 or B12, and to a lesser extent with vitamin B12 to alleviate cold hyperalgesia, but the anticonvulsant drug did not synergize with low dosage vitamin B6 treatment to modify orofacial mechanical hyperalgesia. The latter finding is at variance with the marked synergism towards inhibition of hind paw mechanical hyperalgesia induced by L5/L6 spinal nerve ligation when rats were treated with a combination of gabapentin plus a mixture of B1, B6 and B12 (Reyes-Garcia et al., 2004), or with carbamazepine or gabapentin together with either benfotiamine or vitamin B12 (Mixcoatl-Zecuatl et al., 2008).

Various studies have sought to elucidate the mechanisms underlying the inhibition of neuropathic pain by B vitamins. Alleviation of mechanical hyperalgesia in streptozotocin-induced diabetic rats by a mixture of vitamins B1, B6 and B12, or by vitamin B6 alone was associated with improved sensory nerve conduction velocity (Jolivalt et al., 2009), but no such change was found in rats rendered diabetic with 2,5-hexanedione and treated with vitamin B6 or B12 alone (Misumi et al., 1985). Other possible antihyperalgesic mechanisms of B vitamins include inhibition of the diacylglycerol-protein kinase C (PKC) pathway (Hammes et al., 2003; Sánchez-Ramírez et al., 2006), enhanced cGMP production by guanylylcyclase enzyme (Vesely, 1985) and increased afferent inhibitory control of nociceptive neurons at the spinal cord (Fu et al., 1998). More recently, Song et al. (2009) showed that B1 vitamin treatment reduced neuronal hyperexcitability and lessen alterations of Na^+ currents in injured dorsal root ganglia, and proposed that such mechanisms might contribute to its thermal antihyperalgesic effect. Considering that blockade of voltage-dependent Na^+ channels in hyperexcitable neurons of the dorsal root or trigeminal ganglia has also been implicated in the analgesic effect of anticonvulsant drugs in neuropathic pain (Cardenas et al., 2006; Caviedes and Herranz, 2002; Priest, 2009), the antihyperalgesic synergism between carbamazepine and B vitamins may be the result of their combined action on this common target. However, the differential effects of the vitamins depending on the type of stimulus used

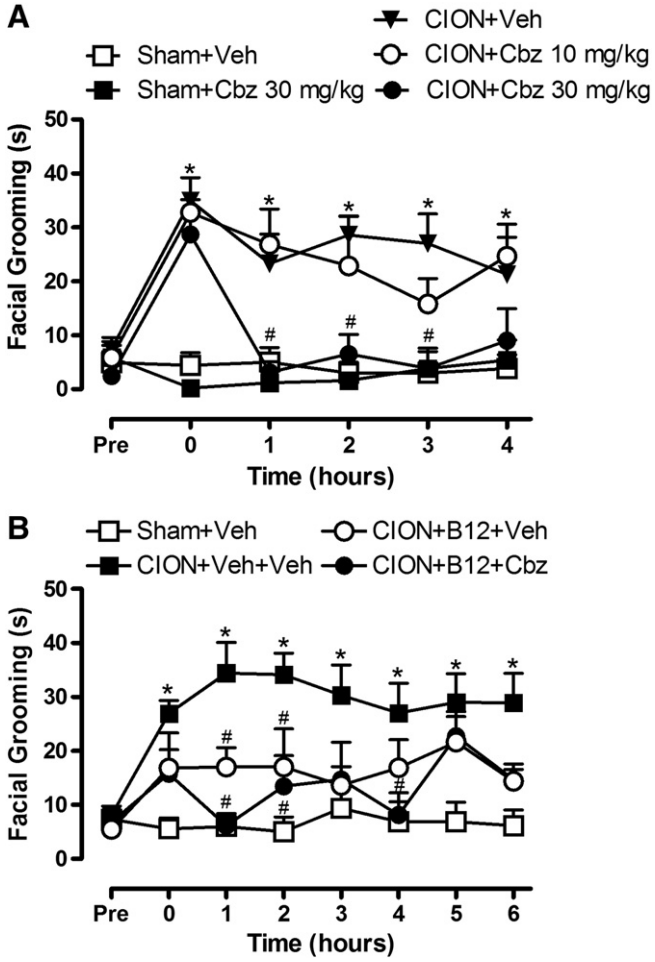

Fig. 6. Effect of carbamazepine alone or in combination with vitamin B12 on cold hyperalgesia induced by CION surgery. Basal cold responsiveness was assessed before CION surgery (Pre) and then again on day 4 after surgery, first prior to (0 h) and then at each hour after the injections of that day (see below). On day 4 after CION, rats received a single injection of carbamazepine (10 and 30 mg/kg, i.p.) or vehicle (1 mL/kg, i.p.; panel A). Alternatively, rats were treated daily with vehicle (saline, 1 mL/kg, s.c.) or vitamin B12 at 1.8 mg/kg/day, for 5 days, starting on the day of CION surgery and on day 4 the last injection was given simultaneously with a injection of either vehicle (1 mL/kg, i.p.) or carbamazepine (10 mg/kg, i.p.; panel B). Values represent means ± SE mean of 5–8 rats. * and # indicate $P<0.05$ when compared to corresponding value of sham- and CION-operated rats treated with vehicle, respectively (Two way ANOVA followed by Duncan test).

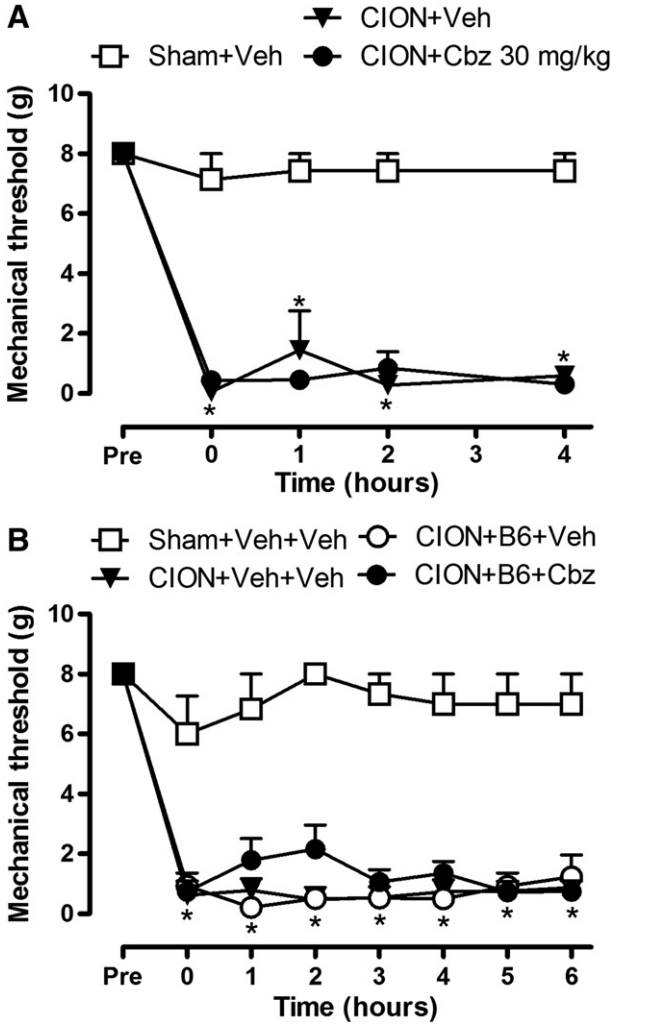

Fig. 7. Effect of carbamazepine alone or in combination with vitamin B6 on mechanical hyperalgesia induced by CION surgery. Basal mechanical responsiveness was assessed before CION surgery (Pre) and then again after surgery, first prior to (0 h) and then at each hour after the injections of that day. On day 20 after CION, rats received a single injection of carbamazepine (30 mg/kg, i.p.) or vehicle (1 mL/kg, i.p.; panel A). In panel B, rats were treated daily with vehicle (saline, 1 mL/kg, s.c.) or vitamin B6 at 18 mg/kg/day, for 5 days, starting on the day 8 after CION surgery and the last injection was given simultaneously with an injection of either vehicle (1 mL/kg, i.p.) or carbamazepine (30 mg/kg, i.p.) on day 12 after CION. Values represent means ± SE mean of 5–7 rats. * Indicate $P<0.05$ when compared to corresponding value of sham-operated rats treated with vehicle (Two way ANOVA followed by Duncan test).

(i.e. cold, heat or mechanical) renders their mechanisms of action even more intriguing and further studies are still necessary to elucidate this issue.

Even if their analgesic mechanisms remain to be elucidated, we regard the pre-clinical and clinical studies with B vitamins as very promising for two main reasons: the low risk of adverse effects related to B vitamins use, and their potential for use as adjuvants in the control of pain. Several experimental studies reported that acute or repeated treatment with high doses of B vitamins did not cause signs of motor disturbance, a common side effect related to anticonvulsants (Medina-Santillan et al., 2004; Mixcoatl-Zecuatl et al., 2008; Reyes-Garcia et al., 2002, 2003, 2004). In fact, neuropathic pain patients treated with gabapentin plus B vitamins showed a delayed appearance of gabapentin-related side effects when compared to those treated with anticonvulsant alone (Medina-Santillan et al., 2004). The present study, alongside others (Caram-Salas et al., 2004, 2006; Granados-Soto et al., 2004; Mixcoatl-Zecuatl et al., 2008; Reyes-Garcia et al., 2002, 2003, 2004), demonstrate that association of B vitamins with drugs of other pharmacological classes, including anticonvulsants, steroidal and non-steroidal anti-inflammatory drugs enables synergism between their analgesic effects, which allows for lowering of doses and fewer side effects.

Conclusion

In conclusion, repeated treatment with vitamins B1, B6 or B12 reduces heat hyperalgesia, cold hyperalgesia (vitamin B12) and mechanical hyperalgesia (vitamin B6) inflicted by CION surgery in rats, a model of trigeminal neuralgia. At lower doses, these vitamins synergize with carbamazepine to provide greater relief of heat and cold hyperalgesia, but not mechanical hyperalgesia. If these findings are reproducible in humans, B vitamins alone or in combination with anticonvulsant drugs could represent a potentially inexpensive and safe long-term approach in the pharmacotherapy of trigeminal neuropathic pain in the clinic.

Conflict of interest statement

The authors declare that there are no conflicts of interest regarding publication of this study.

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